G-protein coupled receptor auto-antibodies in thromboangiitis obliterans (Buerger’s disease) and their removal by immunoadsorption

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Summary

Background: Immunhistopathological and serological data favors an immunopathogenesis of thromboangiitis obliterans (TAO, Buerger’s disease). Autoantibodies seem to play a major role. Immunoadsorption (IA) proved to be therapeutically effective. We focused on agonistic autoantibodies (agAAB) directed against G-protein coupled receptors (GPCR) and proved the hypothesis, that these agAAB might be present in TAO and that a five day course of IA might be able to eliminate these agAAB effectively.

Patients and methods: Between December 2012 and May 2014 11 TAO-patients were treated by IA in a five day course. AgAAB-analysis was performed using specific ELISA techniques.

Results: AgAAB were detected in 9 out of 11 patients (81.8 %). Multiple agAAB were present in 7 patients (63.6 %). A clustering of agAAB directed against loop1 of the adrenergic α1-receptor and the endothelin-A-(ETA)receptor was identified, representing 72.7 % resp. 54.5 % of the patients. AgAAB directed against the angiotensin-1 (AT-1) epitope 1 or 2 were detected in 3 patients and agAAB directed against protease-activated receptor (PAR) loop1/2 were seen in 2 patients. AgAAB directed against ETA-receptor loop 1 never appeared without agAAB directed against α1-receptor loop 1. Immediately after a five day-course of IA agAAB were absent in 81.8 % of the total study group and in 77.8 % of all cases tested positive for agAAB before IA.

Conclusions: AgAAB directed against GPCR were identified in TAO patients with a clustering of agAAB directed against α-1-adrenergic receptor loop 1 and ETA-receptor loop 1. AgAA were eliminated by IA in the majority of cases. We suggest that these agAA play an important role in the pathogenesis of TAO and that their elimination might be responsible for the positive therapeutic effects reported in patients treated with IA.

Key words: Thromboangiitis obliterans, buerger’s disease, immunoadsorption, autoantibodies, g-protein coupled receptor

Zusammenfassung

Agonistische Autoantikörper, die gegen G-Protein gekoppelte Rezeptoren (GPCR) gerichtet sind, bei Patienten mit Thrombangiitis obliterans und ihre Entfernung durch Immunadsorption

Hintergrund: Histopathologische und serologische Befunde sprechen für eine Immunopathogenese der Thromboangiitis obliterans (TAO, Buerger’sche Erkrankung). Autoantikörper scheinen eine wichtige Rolle im Krankheitsgeschehen zu spielen. Es wurden wiederholt positive therapeutische Effekte durch eine Immunadsorption (IA) gezeigt. Wir analysierten agonistische Autoantikörper (agAAK), die gegen G-Protein gekoppelte Rezeptoren (GPCR) gerichtet sind und analysierten, ob diese durch eine IA effektiv beseitigt werden können.


Ergebnisse: AgAAK wurden bei 9 von 11 Patienten (81.8 %) gefunden. Multiple agAAK waren bei 7 Patienten (63.6 %) nachweisbar. Darüber hinaus wurde ein agAAK-Cluster entdeckt, das sich gegen Loop 1 des adrenergen α1-Rezeptors und Loop 1 des Endothelin A (ETA)-Rezeptors richtet, und sich bei 72.7 % respektive 54.5 % der TAO-Patienten nachweisen ließ. AgAAK gegen Epitop 1 oder 2 des Angiotensin-1-Rezeptors wurden bei 3 Patienten, agAAK gegen Loop1/2 des Proteinase-aktivierten Rezeptor (PAR) wurden bei 2 Patienten nachgewiesen. AgAAG gegen den ETA-Rezeptor traten niemals ohne gleichzeitigen Nachweis von agAAG gegen den α1-adrenergen Rezeptor auf und waren immer gegen Loop1 gerichtet. Unmittelbar nach Beendigung der IA ließen sich bei 81.8 % aller Patienten und bei 77.8 % aller Fälle mit positiven agAAG vor der IA keine agAAG mehr nachgewiesen.

Schlussfolgerungen: Wir konnten bei Patienten mit aktiver TAO agAAG gegen GPCR und darüber hinaus ein Antikörper-Cluster nachweisen, das sich bevorzugt gegen Loop1 des α1-adrenergen Rezeptors und Loop1 des ETA-Rezeptors richtet. Die agAAG wurden mehrheitlich erfolgreich durch die IA eliminiert. Wir sind der Auffassung, dass gegen GPCR-gerichtete agAAG eine wichtige Rolle in der Pathogenese der TAO spielen und ihre Elimination für die publizierten positiven Effekte der IA verantwortlich sein könnten.
Introduction

Thromboangiitis obliterans (TAO; Buerger’s disease) is a inflammatory disease of unknown origin affecting small and medium sized arteries and veins of young tobacco users threatening limbs by vessel occlusions with mononuclear cell rich thrombi [1 – 4]. Endothelial activation and proliferation as well as the presence of immunocompetent cells together with deposition of immunoglobulins und complement factors alongside the internal elastic membrane of the vessel wall argue for an immunopathogenesis of TAO [5 – 11]. Furthermore, in the sera of TAO-patients various kinds of autoantibodies have been described including antienothelial antibodies, antibodies directed against elastin and collagen, anticardiolipin-antibodies, ANCA, and ANA [12 – 15].

Agonistic autoantibodies (agAAB) reacting with epitopes localized at the extracellular loops of G-protein coupled receptors (GPCR) are increasingly recognized as modulators of various cardiovascular pathologies [16 – 19]. In this context, agAAB directed against α1-adrenergic receptor [20 – 26], β1-adrenergic receptor [27 – 29], β2-adrenergic receptor [23, 26] endothelin A receptor [20] and angiotensin-1 receptor [22, 26, 30, 31] have been described. Binding of agAAB to the receptor leads to activation of downstream signaling cascades [32]. Deviant to physiological GPCR-agonists activation of receptor pathways by agAAB may result in a prolonged and unphysiological activation of postreceptor action [28].

The pathogenic potential of circulating agAAB has already been demonstrated in animal models as well as in clinical studies [25, 33 – 36].

With this study we tested the hypothesis, that agAAB directed against GPCR are present in the sera of TAO patients and that they might be removed sufficiently by a five day course of immunoadsorption (IA). Subsequently, we present preliminary results of an ongoing project.

Patients and methods

From December 2012 to May 2014 thirteen patients presumably suffering from TAO were treated with IA in our institution. Diagnosis of TAO was made according to the criteria published by Olin et al. and Shionoya [37, 38]. Diagnosis of TAO was corrected retrospectively in two patients due to the finding of high concentrations of Lp(a) in one case and the detection of atherosclerotic coronary artery disease in another, leaving eleven patients for final analysis.

Patients’ characteristics are shown in Table I. All were cigarette smokers; two had recently stopped smoking, while two admitted additional cannabis abuse.

All patients had received best medical treatment before IA including intravenous iloprost-infusions. Four had undergone minor amputations, whereas one patient was already major amputated.

IA was performed during a five day course as described elsewhere [39, 40]. Alternatively, Globaffin®-adsorbers with a synthetic Peptid-GAM® ligand coupled to a sepharose matrix (Fresenius Medical Care AG & Co. KGaA, Bad Homburg v.d. Höhe, Germany) were used in all treatment sessions in this series.

Blood samples were drawn each day before and after IA from a central venous line placed immediately before the first treatment. Autoantibody analysis was performed using peptides corresponding to the first and/or second extracellular loop of following GPCR: α1, Endothelin A, Angiotensin II Type 1, protease activated receptor (PAR) 1/2. Peptides were coupled to pre-blocked streptavidin-coated 96-well plates (Perbio Science, Bonn, Germany). Patient serum was added in a 1:100 dilution and incubated for 60 min. As detection antibody a horseradish peroxidase conjugated anti-human IgG antibody was used (Dianova, Hamburg, Germany). Antibody binding was visualized by the 1-Step Ultra TMB ELISA (Perbio Science, Bonn, Germany). The absorbance was measured at 450 nm with a SLT Spectra multiplate reader (TECAN, Crailsheim, Germany).

Results

Results of agAAB-testing before and after IA are summarized in table II. AgAAB were identified in 9 out of 11 patients (81.8 %), while multiple agAAB were present in 7 patients (63.6 %). Three agAAB were present in 3 patients (27.3 %), while 2 agAAB were detected in 5 patients (45.5 %). A clustering of α1-adrenergic receptor and endothelin A (ETA) receptor agAAB was identified, representing 72.7 % resp. 54.5 % of the TAO-patients. Six patients (54.5 %) were positive for both of these two agAAB. AgAA directed against the ETA-receptor never appeared without agAAB directed against the α1-receptor. Both were exclusively directed to the extracellular receptor-loop1.

AgAAB directed against the angiotensin-1 (AT-1) epitope 1 or 2 were positive in 3 patients and agAAB directed against protease-activated receptor (PAR) loop1/2 were seen in 2 patients. Immediately after the end of the five-day course agAAB were negative in 9 patients (81.8 %). Focusing exclusively on those cases with positive agAAB before IA (n = 9) the elimination rate at the end of the last treatment session was 77.8 %. Majoritarily, agAAB were eliminated after the first three sessions. However, persistence of at least one agAAB was observed in 2 patients, while reoccurrence of one agAAB from day-
Table I: Clinical characteristics of the study group

<table>
<thead>
<tr>
<th>Patient, initials</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Duration of disease (y)</th>
<th>Smoking status (cigarettes)</th>
<th>Ischemic rest pain, visual analog pain scale result</th>
<th>Acral cyanosis</th>
<th>Active ulcer/gangrene/wounds</th>
<th>Former amputation</th>
<th>Thrombo-phlebitis</th>
<th>Raynaud’s phenomenon</th>
<th>Other diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 NA</td>
<td>40</td>
<td>male</td>
<td>2</td>
<td>active</td>
<td>yes, VAS 7</td>
<td>left forefoot</td>
<td>D ped I, II left</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>pulmonary embolism 2008</td>
</tr>
<tr>
<td>2 LH</td>
<td>53</td>
<td>male</td>
<td>3</td>
<td>active</td>
<td>yes, VAS 6</td>
<td>fingers right hand</td>
<td>D II right</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>acute gastritis and duodenitis, extirpated basalioma, mild hypercholesterolemia</td>
</tr>
<tr>
<td>3 MM</td>
<td>48</td>
<td>female</td>
<td>4</td>
<td>active</td>
<td>yes, VAS 9</td>
<td>left forefoot</td>
<td>left forefoot</td>
<td>D IV and V left as well as partial amputations D I, II ped left</td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>4 NC</td>
<td>39</td>
<td>male</td>
<td>2</td>
<td>former</td>
<td>yes, VAS 7</td>
<td>–</td>
<td>D I right</td>
<td>D I ped left</td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>5 RT</td>
<td>42</td>
<td>male</td>
<td>2</td>
<td>former</td>
<td>yes, VAS 9</td>
<td>right forefoot</td>
<td>Diphosphatase, dehiscence amputation wounds</td>
<td>Dig V transmetatarsal left, dig ped II left</td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>6 RD</td>
<td>20</td>
<td>male</td>
<td>1.5</td>
<td>active</td>
<td>yes, VAS 8</td>
<td>bilateral forefoot</td>
<td>left forefoot, toes right</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>additional cannabis abuse</td>
</tr>
<tr>
<td>7 VK</td>
<td>52</td>
<td>male</td>
<td>4</td>
<td>active</td>
<td>yes, VAS 7</td>
<td>bilateral forefeet</td>
<td>toes right foot</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>Occluded distal arterial bypass</td>
</tr>
<tr>
<td>8 BS</td>
<td>26</td>
<td>male</td>
<td>1</td>
<td>active</td>
<td>yes, VAS 8</td>
<td>bilateral forefeet</td>
<td>D I ped left</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>additional cannabis abuse, penicillin allergy, seronegative spondylarthritis</td>
</tr>
<tr>
<td>9 FB</td>
<td>50</td>
<td>male</td>
<td>2</td>
<td>active</td>
<td>yes, VAS 7</td>
<td>–</td>
<td>D II, V ped left</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>acute forefoot-infection; alcoholism, mild hypertension</td>
</tr>
<tr>
<td>10 BR</td>
<td>27</td>
<td>male</td>
<td>1</td>
<td>active</td>
<td>yes, VAS 8</td>
<td>right forefoot</td>
<td>D I ped right</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>–</td>
</tr>
<tr>
<td>11 HG</td>
<td>51</td>
<td>male</td>
<td>3</td>
<td>former</td>
<td>yes VAS 9</td>
<td>–</td>
<td>amputation wound left forefoot</td>
<td>right lower leg, recently left forefoot</td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>∑</td>
<td>40.7</td>
<td>n : f = 10:1</td>
<td>2.3</td>
<td>8 active, 3 former smokers</td>
<td>rest pain 11/11, VAS (Pain-scale) 7.7</td>
<td>8/11 (73%)</td>
<td>11/11</td>
<td>4/11 (36%) minor 1/11 (9%) major</td>
<td>1/11 (9%)</td>
<td>4/11 (36%)</td>
<td>–</td>
</tr>
</tbody>
</table>
to-day was observed in another case. Thus, effective total elimination rate obtained with our protocol presumably adds up to 66.6%.

Discussion

AgAA directed against GPCR are increasingly recognized as modulators of various cardiovascular pathologies [16 –19] including systemic and pulmonary arterial hypertension [20, 24, 25, 41], idiopathic dilated cardiomyopathy [27, 29, 33, 42], type 2 diabetes [22], dementia [23], pre-eclampsia [19, 31], vascular necrotic kidney graft rejection [30, 43] as well as in scleroderma angiopathy [44]. The prevalence of agAAB in cardiovascular diseases and diabetes varies grossly from 30 to 60%. Analyzing preliminary data of an ongoing project we detected agAAB against GPCR in nine out of eleven patients (81.8%) with active Buerger’s disease and multiple agAAB against GPCR in 7 of them (63.6%). Furthermore, we were able to identify clustering of agAAB binding to the extracellular receptor loop1 of the α1-adrenergic and the ETA-receptor, while agAAB directed against loop2 of these two receptors were not detectable. To our knowledge, this is the first report addressing this topic and identifying this agAAB-pattern in patients with active TAO. Notably, after a five-day course of IA all cases tested positive for agAAB and identifying this agAAB-pattern grossly from 30 to 60%.

We assume that the elimination of the agAAB identified by us ameliorates vasospasm and improves microcirculation. The effects might explain published clinical effects of IA in TAO that cover marked improvement of pain, improvement of digital pulse curves, steep incline of tcpO2-levels, decrease of tcCO2-levels, and ulcer healing [39, 40]. Nevertheless, the exact pathophysiologic role of GPCR-AAB in TAO has yet to be defined experimentally. Further studies will also have to re-produce our results in larger cohorts and address the time course as well as clinical relevance of re-occurrences of agAAB after IA.

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Conflicts of interest

Autoantibody analysis was supported by Fresenius Medical Care AG & Co. KGaA, Bad Homburg v.d. Höhe, Germany.

References


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